

(19) World Intellectual Property
Organization
International Bureau



07 JAN 2005



(43) International Publication Date
15 January 2004 (15.01.2004)

PCT

(10) International Publication Number
WO 2004/005535 A3

(51) International Patent Classification⁷: **C12Q 1/48**,
C07K 14/245, C12N 15/31, 15/01, 15/63, C12Q 1/02,
C07K 14/195, 16/12

(21) International Application Number:
PCT/EP2003/008209

(22) International Filing Date: 9 July 2003 (09.07.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02 08 636 9 July 2002 (09.07.2002) FR

(71) Applicant (for all designated States except US): MUTA-
BILIS [FR/FR]; 156, Avenue Vaugirard, F-75730 PARIS
CEDEX 15 (FR).

(72) Inventor; and

(75) Inventor/Applicant (for US only): ESCAICH, Sonia
[FR/FR]; 13, Rue de Toul, F-75012 PARIS (FR).

(74) Agents: PEAUCELLE, Chantal et al.; Cabinet ARMEN-
GAUD AINE, 3, Avenue Bugeaud, F-75116 PARIS (FR).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:
18 November 2004

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: PATHOGENICITY PROTEINS WHICH CAN BE USED AS TARGETS FOR DEVELOPING MEANS FOR PRE-
VENTING AND CONTROLLING BACTERIAL INFECTIONS

(57) Abstract: The invention relates to a method for identifying and selecting a gene required for the proliferation *in vivo* of a pathogenic microorganism, comprising :- using a strain of the pathogenic microorganism, - generating mutants for inactivation in the genes encoding these factors, - determining the virulence of these mutants on an experimental model of infection, and their effect on enteric colonization in an axenic mouse model, and- selecting the bacterial genes essential for resistance to serum *in vitro*, and essential, in the host, for dissemination in the serum. Application to the screening of compounds which inhibit the products of the genes identified, and to the inhibition *in vitro* of the proliferation of a pathogenic microorganism in serum.



WO 2004/005535 A3

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/08209

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/48 C07K14/245 C12N15/31 C12N15/01 C12N15/63
C12Q1/02 C07K14/195 C07K16/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE, CHEM ABS Data, Sequence Search, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>YETHON J A ET AL: "Involvement of waaY, waaQ and waaP in the modification of E. coli lipopolysaccharide and their role in the formation of a stable outer membrane" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 273, no. 41, 9 October 1998 (1998-10-09), pages 26310-26316, XP002118875 ISSN: 0021-9258 the whole document</p> <p style="text-align: center;">----- -/--</p>	5-16, 18, 19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

30 July 2004

Date of mailing of the international search report

26 AUG 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Niemann, F

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HEINRICHS DAVID E ET AL: "Molecular basis for structural diversity in the core regions of the lipopolysaccharides of Escherichia coli and Salmonella enterica." MOLECULAR MICROBIOLOGY, vol. 30, no. 2, October 1998 (1998-10), pages 221-232, XP002118873 ISSN: 0950-382X the whole document</p>	5-16,18,19
X	<p>WO 99/66049 A (HEINRICHS DAVID E ;UNIV GUELPH (CA); WHITFIELD CHRIS (CA); YETHON) 23 December 1999 (1999-12-23) the whole document</p>	5-16,18,19
A	<p>BURNS STACY M ET AL: "Comparison of loss of serum resistance by defined lipopolysaccharide mutants and an acapsular mutant of uropathogenic Escherichia coli 075:K5." INFECTION AND IMMUNITY, vol. 66, no. 9, 1998, pages 4244-4253, XP002250632 ISSN: 0019-9567 cited in the application the whole document</p>	
A	<p>RUSSO THOMAS A ET AL: "Loss of the O4 antigen moiety from the lipopolysaccharide of an extraintestinal isolate of Escherichia coli has only minor effects on serum sensitivity and virulence in vivo." INFECTION AND IMMUNITY, vol. 63, no. 4, 1995, pages 1263-1269, XP002250633 ISSN: 0019-9567 cited in the application the whole document</p>	
A	<p>HONG MEI ET AL: "Effect of mutations in Shigella flexneri chromosomal and plasmid-encoded lipopolysaccharide genes on invasion and serum resistance." MOLECULAR MICROBIOLOGY, vol. 24, no. 4, 1997, pages 779-791, XP008020612 ISSN: 0950-382X abstract</p>	

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MERINO SUSANA ET AL: "Cloning and sequencing of the Klebsiella pneumoniae 05 wb gene cluster and its role in pathogenesis." INFECTION AND IMMUNITY, vol. 68, no. 5, May 2000 (2000-05), pages 2435-2440, XP002250634 ISSN: 0019-9567 the whole document -----	
A	RAUTEMAA RIINA ET AL: "Complement-resistance mechanisms of bacteria." MICROBES AND INFECTION, vol. 1, no. 10, August 1999 (1999-08), pages 785-794, XP002250635 ISSN: 1286-4579 cited in the application the whole document -----	
A	SHEA JACQUELINE E ET AL: "Signature-tagged mutagenesis in the identification of virulence genes in pathogens." CURRENT OPINION IN MICROBIOLOGY, vol. 3, no. 5, October 2000 (2000-10), pages 451-458, XP002250636 ISSN: 1369-5274 the whole document -----	
X	DATABASE UNIPROT 1 May 1999 (1999-05-01), "WaaC" XP002284637 Database accession no. Q9ZITI1 the whole document -----	5-16,18, 19
X	DATABASE UNIPROT 1 May 2000 (2000-05-01), "WaaQ" XP002284638 Database accession no. Q9R9D5 the whole document -----	5-16,18, 19
X	DATABASE UNIPROT 1 May 2000 (2000-05-01), "WaaP" XP002284639 Database accession no. Q9R9D6 the whole document -----	5-16,18, 19
X	DATABASE UNIPROT 1 May 2000 (2000-05-01), "WaaG" XP002284640 Database accession no. Q9R2L8 the whole document -----	5-16,18, 19
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE UNIPROT 1 October 1994 (1994-10-01), "RFAF" XP002284641 Database accession no. P37692 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 May 1999 (1999-05-01), "WaaO" XP002284642 Database accession no. Q9ZIS5 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 May 1992 (1992-05-01), "GalU" XP002284643 Database accession no. P25520 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 June 1994 (1994-06-01), "PGMU" XP002284644 Database accession no. P36938 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 February 1995 (1995-02-01), "KpsD" XP002284645 Database accession no. Q03961 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 November 1996 (1996-11-01), "Neud" XP002284646 Database accession no. Q46674 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 July 1993 (1993-07-01), "GmhB" XP002284647 Database accession no. P31546 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 1 November 1990 (1990-11-01), "RFAD" XP002284648 Database accession no. P17963 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE UNIPROT 15 July 1999 (1999-07-15), "rfaE" XP002284649 Database accession no. P76658 the whole document</p> <p>-----</p>	5-16,18, 19
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE UNIPROT 1 March 2002 (2002-03-01), "DltD" XP002284650 Database accession no. Q8VM64 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>DATABASE GENESEQ 2 July 2002 (2002-07-02), "DLTA" XP002284651 Database accession no. ABP30484 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>KNEIDINGER BERND ET AL: "Biosynthesis pathway of ADP-L-glycero-beta-D-manno-heptose in Escherichia coli" JOURNAL OF BACTERIOLOGY, vol. 184, no. 2, January 2002 (2002-01), pages 363-369, XP002284579 ISSN: 0021-9193 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>KNEIDINGER BERND ET AL: "Biosynthesis of nucleotide-activated D-glycero-D-manno-heptose" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 24, 15 June 2001 (2001-06-15), pages 20935-20944, XP002284580 ISSN: 0021-9258 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>SISTI FEDERICO ET AL: "In vitro and in vivo characterization of a Bordetella bronchiseptica mutant strain with a deep rough lipopolysaccharide structure" INFECTION AND IMMUNITY, vol. 70, no. 4, April 2002 (2002-04), pages 1791-1798, XP002284581 ISSN: 0019-9567 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>YETHON JEREMY A ET AL: "Purification and characterization of Waap from Escherichia coli, a lipopolysaccharide kinase essential for outer membrane stability" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 8, 23 February 2001 (2001-02-23), pages 5498-5504, XP002284582 ISSN: 0021-9258 the whole document</p> <p>-----</p>	5-16,18, 19

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>YETHON JEREMY A ET AL: "Salmonella enterica serovar typhimurium waaP mutants show increased susceptibility to polymyxin and loss of virulence in vivo" INFECTION AND IMMUNITY, vol. 68, no. 8, August 2000 (2000-08), pages 4485-4491, XP002284583 ISSN: 0019-9567 the whole document</p>	5-16,18, 19
X	<p>GENEVAUX PIERRE ET AL: "Identification of Tn10 insertions in the rfaG, rfaP, and galU genes involved in lipopolysaccharide core biosynthesis that affect Escherichia coli adhesion" ARCHIVES OF MICROBIOLOGY, vol. 172, no. 1, July 1999 (1999-07), pages 1-8, XP002284584 ISSN: 0302-8933 the whole document</p>	5-16,18, 19
A	<p>NGELEKA MUSANGU ET AL: "Characterization of a polysaccharide capsular antigen of septicemic Escherichia coli 0115: K "V165": F165 and evaluation of its role in pathogenicity" INFECTION AND IMMUNITY, vol. 60, no. 12, 1992, pages 5048-5056, XP002284585 ISSN: 0019-9567 the whole document</p>	
X	<p>DAINES DAYLE A ET AL: "NeuD plays a role in the synthesis of sialic acid in Escherichia coli K1" FEMS MICROBIOLOGY LETTERS, vol. 189, no. 2, 15 August 2000 (2000-08-15), pages 281-284, XP002284613 ISSN: 0378-1097 the whole document</p>	5-16,18, 19
X	<p>YETHON JEREMY A ET AL: "Mutation of the lipopolysaccharide core glycosyltransferase encoded by waaG destabilizes the outer membrane of Escherichia coli by interfering with core phosphorylation" JOURNAL OF BACTERIOLOGY, vol. 182, no. 19, October 2000 (2000-10), pages 5620-5623, XP002284615 ISSN: 0021-9193 the whole document</p>	5-16,18, 19

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>NESPER JUTTA ET AL: "Characterization of <i>Vibrio cholerae</i> O1 El Tor galU and galE mutants: Influence on lipopolysaccharide structure, colonization, and biofilm formation"</p> <p>INFECTION AND IMMUNITY, vol. 69, no. 1, January 2001 (2001-01), pages 435-445, XP002284616 ISSN: 0019-9567 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>ABACHIN ERIC ET AL: "Formation of D-alanyl-lipoteichoic acid is required for adhesion and virulence of <i>Listeria monocytogenes</i>"</p> <p>MOLECULAR MICROBIOLOGY, vol. 43, no. 1, January 2002 (2002-01), pages 1-14, XP002284617 ISSN: 0950-382X the whole document</p> <p>-----</p>	5-16,18, 19
A	<p>MARTINDALE J ET AL: "Genetic analysis of <i>E. Coli</i> K1 gastrointestinal colonization"</p> <p>MOLECULAR MICROBIOLOGY, vol. 37, no. 6, September 2000 (2000-09), pages 1293-1305, XP002284618 ISSN: 0950-382X the whole document</p> <p>-----</p>	
X	<p>CHANG HWAN-YOUNG ET AL: "Virulence and outer membrane properties of a galU mutant of <i>Klebsiella pneumoniae</i> CG43"</p> <p>MICROBIAL PATHOGENESIS, vol. 20, no. 5, 1996, pages 255-261, XP002284619 ISSN: 0882-4010 the whole document</p> <p>-----</p>	5-16,18, 19
X	<p>REGNI CATHERINE ET AL: "Crystal structure of PMM/PGM: An enzyme in the biosynthetic pathway of <i>P. aeruginosa</i> virulence factors"</p> <p>STRUCTURE (CAMBRIDGE), vol. 10, no. 2, February 2002 (2002-02), pages 269-279, XP002284620 ISSN: 0969-2126 the whole document</p> <p>-----</p>	5-16,18, 19

-/--

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TANG C ET AL: "PATHOGEN VIRULENCE GENES - IMPLICATIONS FOR VACCINES AND DRUG THERAPY" BRITISH MEDICAL BULLETIN, CHURCHILL LIVINGSTONE, LONDON, GB, vol. 55, no. 2, 1999, pages 387-400, XP008008098 ISSN: 0007-1420 the whole document	
A	WO 00/28038 A (CLARKE ENDA ELIZABETH ; CROOKE HELEN RACHEL (GB); EVEREST PAUL HOWARD) 18 May 2000 (2000-05-18) the whole document	
A	MOXON R ET AL: "CHALLENGE OF INVESTIGATING BIOLOGICALLY RELEVANT FUNCTIONS OF VIRULENCE FACTORS IN BACTERIAL PATHOGENS" PHILOSOPHICAL TRANSACTIONS. ROYAL SOCIETY OF LONDON. BIOLOGICAL SCIENCES, ROYAL SOCIETY, LONDON, GB, vol. 355, no. 1397, 25 May 2000 (2000-05-25), pages 643-656, XP001007306 ISSN: 0962-8436 the whole document	
A	US 6 020 121 A (BOGGS AMY ET AL) 1 February 2000 (2000-02-01) the whole document	
X	WO 00/44906 A (ELITRA PHARMACEUTICALS INC) 3 August 2000 (2000-08-03) sequence 165	5-16,18, 19
X	VALVANO M A ET AL: "The rfaE gene from Escherichia coli encodes a bifunctional protein involved in biosynthesis of the lipopolysaccharide core precursor ADP-L-glycero-D-manno-heptose" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 182, no. 2, January 2000 (2000-01), pages 488-497, XP000926030 ISSN: 0021-9193 the whole document	5-16,18, 19
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>LEE N G ET AL: "Molecular cloning and characterization of the nontypable Haemophilus influenzae-2019 rfaE gene required for lipopolysaccharide biosynthesis"</p> <p>INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 63, no. 3, 1995, pages 818-824, XP000953326</p> <p>ISSN: 0019-9567</p> <p>the whole document</p>	5-16,18,19
X	<p>-----</p> <p>CIESLEWICZ M ET AL: "THERMOREGULATION OF KPSF, THE FIRST REGION 1 GENE IN THE KPS LOCUS FOR POLYSIALIC ACID BIOSYNTHESIS IN ESCHERICHIA COLI K1"</p> <p>JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 178, no. 11, June 1996 (1996-06), pages 3212-3220, XP000877094</p> <p>ISSN: 0021-9193</p> <p>the whole document</p>	5-16,18,19
X	<p>-----</p> <p>BOYD E F & HARTL D L: "Chromosomal regions specific to pathogenic isolates of Escherichia coli have a phylogenetically clustered distribution"</p> <p>JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 180, no. 5, March 1998 (1998-03), pages 1159-1165, XP002133065</p> <p>ISSN: 0021-9193</p> <p>the whole document</p>	5-16,18,19
X	<p>-----</p> <p>AUSTIN A E ET AL: "Genetic analysis of lipopolysaccharide core biosynthesis by Escherichia coli k12 insertion mutagenesis of the RFA locus"</p> <p>JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 172, no. 9, September 1990 (1990-09), pages 5312-5325, XP000926028</p> <p>ISSN: 0021-9193</p> <p>the whole document</p>	5-16,18,19
X	<p>-----</p> <p>STOJILJKOVIC I ET AL: "CLONING AND CHARACTERIZATION OF THE NEISSERIA MENINGITIDIS RFAC GENE ENCODING ALPHA-1,5 HEPTOSYLTRANSFERASE I"</p> <p>FEMS MICROBIOLOGY LETTERS, AMSTERDAM, NL, vol. 151, no. 1, 1997, pages 41-49, XP001007307</p> <p>ISSN: 0378-1097</p> <p>the whole document</p> <p>-----</p>	5-16,18,19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/08209

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-4, 17, 20
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4,17,20

Present claims 1-2 relate to a method for identifying and selecting a gene required for the proliferation in vivo of a pathogenic microorganism, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for none of such methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible.

Present claims 3-4 relate to mutant nucleic acids for inactivation of the virulence genes as implemented in the method according to claim 1 or 2 and to mutant nucleic acids which are sensitive to serum avirulent in mice model and able to colonize gut of axenic mice, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for none of such mutant nucleic acids. Furthermore the meaning of claims 3-4 is unclear. In the present case, the claims so lack support and clarity, and the application so lacks disclosure, that a meaningful search is impossible.

Present claims 17,20 relate to a compound defined by reference to a desirable characteristic or property, namely being capable of inhibiting the activity, or of reducing the amount, of pathogenicity or virulence target.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for none of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/08209

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9966049	A	23-12-1999	AU 4253299 A	05-01-2000
			CA 2330544 A1	23-12-1999
			WO 9966049 A2	23-12-1999
			US 6570006 B1	27-05-2003
WO 0028038	A	18-05-2000	AU 773003 B2	13-05-2004
			AU 1060500 A	29-05-2000
			BR 9915165 A	04-12-2001
			CA 2348699 A1	18-05-2000
			CN 1330714 T	09-01-2002
			EP 1129196 A2	05-09-2001
			WO 0028038 A2	18-05-2000
			JP 2002529091 T	10-09-2002
			NZ 511366 A	26-03-2004
US 6020121	A	01-02-2000	AU 7168696 A	17-04-1997
			WO 9711690 A2	03-04-1997
WO 0044906	A	03-08-2000	AU 3584500 A	18-08-2000
			CA 2360085 A1	03-08-2000
			EP 1149166 A2	31-10-2001
			EP 1178052 A2	06-02-2002
			JP 2002535007 T	22-10-2002
			NZ 512835 A	27-02-2004
			WO 0044906 A2	03-08-2000
			US 6720139 B1	13-04-2004
			US 2002045592 A1	18-04-2002

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.